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Placental glycogen stores and fetal growth: insights from genetic mouse models

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Key words

Mouse placenta, glycogen, glycogen cells, fetal growth

Abbreviations

C-TGC	Canal Trophoblast Giant Cell (also C-)
Ch-TGC	Channel Trophoblast Giant Cell (also Ch-)
Dec	Decidua
E	Embryonic day
ExE	Extraembryonic ectoderm
EPC	Ectoplacental cone
FGR	Fetal growth restriction
GlyT	Glycogen trophoblast
ICM	Inner cell mass
Jz	Junctional zone
Lz	Labyrinth zone
PAS	Periodic acid Schiff
PE	Preeclampsia
P-TGC	Parietal Trophoblast Giant Cell (also P-)
S-TGC	Sinusoidal Trophoblast Giant Cell (also S-)
SpA-TGC	Spiral Artery-associated Trophoblast Giant Cell (also SpA-)
SpT	Spongiotrophoblast

Abstract

The placenta performs a range of crucial functions that support fetal growth during pregnancy, including facilitating the supply of nutrients and gases to the fetus, removal of waste products from the fetus, and the endocrine modulation of maternal physiology. The placenta also stores glucose in the form of glycogen, the function of which remains unknown. Aberrant placental glycogen storage in humans is associated with maternal diabetes during pregnancy and pre-eclampsia, thus linking placental glycogen storage and metabolism to pathological pregnancies. To understand the role of placental glycogen in normal and complicated pregnancies, we must turn to animal models. Over 40 targeted mutations in mice demonstrate defects in placental cells that store glycogen and suggest that placental glycogen represents a source of readily mobilised glucose required during periods of high fetal demand. However, direct functional evidence is currently lacking. Here, we evaluate these genetic mouse models with placental phenotypes that implicate glycogen trophoblast cell differentiation and function to illuminate the common molecular pathways that emerge and to better understand the relationship between placental glycogen and fetal growth. We highlight current limitations to exploring key questions regarding placental glycogen storage and metabolism and define how to experimentally overcome these constraints.

Introduction

The placenta is a unique organ that exists only during pregnancy to perform a diverse range of functions that collectively support optimal fetal growth whilst maintaining maternal well-being. Arguably, the principal role of the placenta is to mediate the supply of oxygen and nutrients from the mother to fetus (Burton and Fowden, 2015). The placenta also removes waste products from the fetal circulation and forms a physical barrier that affords some protection against certain pathogens and toxins (Gude et al., 2004). Furthermore, the placenta also synthesises and secretes hormones into the mother's blood to adapt maternal physiology and behaviour, ultimately to ensure that the pregnancy is sustained, and fetal growth supported (Napso et al., 2018). In addition to facilitating glucose transport, the placenta metabolises glucose for its own use and stores it in the form of the multi-branched polysaccharide glycogen. The purpose of placental glycogen stores in normal pregnancy remains unclear. The most widely accepted theory is that placental glycogen stores ensure that fetal glucose supply is maintained at times of maximal demand, such as during late gestation (Coan et al., 2006, Barash and Shafrir, 1990). However, direct experimental evidence to support such a function is currently lacking.

Placental glycogen in humans

Despite the earliest reference to glycogen storage in the human placenta dating to the early 20th Century (Driessen, 1907), understanding its role during normal or complicated pregnancies has remained an elusive goal. In the human placenta, glycogen is predominantly found in the cytotrophoblast cells (Wislocki and Bennett, 1943), with the highest levels in the distal cytotrophoblast closest to the decidua (Georgiades et al., 2002). In uncomplicated pregnancies, placental glycogen content peaks towards the end of the first trimester before declining towards term (Villee, 1953, Robb and Hytten, 1976, Boyd, 1959). Aberrant glycogen storage was reported in gestational diabetes (Heijkenskjold and Gemzell, 1957, Diamant et al., 1982, Gabbe et al., 1972, Desoye et al., 1992, Gheorman et al., 2013) and preeclampsia (PE) (Arkwright et al., 1993, Tsoi et al., 2003), suggesting a possible role in the pathogenesis of these two common pregnancy complications. The relevance of aberrant glycogen storage in the context of human pregnancy complications has been reviewed elsewhere (Akison et al., 2017). However, one hypothesis suggests that in diabetic pregnancies the placenta stores

excess glucose as glycogen to protect the fetus from maternal hyperglycaemia, thus limiting fetal overgrowth (Desoye et al., 2002).

Considerable constraints limit our ability to explore placental glycogen function in humans, such as the lack of available tissue at the appropriate developmental stage. Accordingly, it is necessary to turn to the mouse model, which benefits from large litter sizes, a short gestation period that allows for the investigation of early pregnancy, and a similar genome to humans that is easily manipulated. There are 47 known mouse models with targeted mutations that display phenotypes associated with the trophoblast cell subtype that stores glycogen. A common feature among these placental mutants is that fetal growth is co-ordinately affected. Therefore, use of these mutant mouse lines will help to elucidate the normal physiological role of placental glycogen and may provide further insight to the relevance of placental glycogen in the human placental during normal and complicated pregnancies.

Placental development in the mouse

The mouse is a particularly useful model for the human placenta, since they are both haemochorial, are architecturally similar and express many of the same genes that regulate placental development and function (Rossant and Cross, 2001). In particular, imprinted genes, which are characterised by expression derived wholly, or predominantly, from one or other parental allele, exert significant influence on placental development in both humans (Frost and Moore, 2010) and mice (Tunster et al., 2013). Furthermore, the mouse placenta also accumulates glycogen stores in a specialised trophoblast cell subtype called the glycogen trophoblast (GlyT), some of which interact intimately with the maternal decidua (Coan et al., 2006, Bouillot et al., 2005, Rampon et al., 2005). For this reason, GlyT cells share similarities with human cytotrophoblast cells, and establish the mouse as an ideal model in which to elucidate the function(s) of placental glycogen stores.

In the mouse, GlyT appear in cell clusters in the junctional zone (Jz) of the mature placenta, before a proportion of these cells invade the maternal decidua to congregate around maternal spiral arteries (Adamson et al., 2002). When considering how defects in placental glycogen storage and metabolism emerge, it is important to consider when GlyT first arise during placental development, and whether defective GlyT differentiation is the primary cause of altered placental glycogen content. Development of the mouse placenta

under normal circumstances has been extensively reviewed elsewhere (Watson and Cross, 2005, Rossant and Cross, 2001). Briefly, by implantation at embryonic day (E) 4.5, the mouse blastocyst comprises a layer of trophectoderm that surrounds the inner cell mass (ICM). The trophectoderm cells immediately adjacent to the ICM proliferate to give rise to two main trophoblast progenitor populations: the ectoplacental cone (EPC), which will differentiate to yield cells of the centrally located junctional zone (Jz) that is predominantly endocrine in function; and the extraembryonic ectoderm (ExE), which will eventually give rise to the labyrinth zone (Lz) where, when vascularised by fetal capillaries, nutrient, gas and waste exchange between maternal and fetal blood circulations occurs (Fig 1).

To date, nine trophoblast cell subtypes are known to differentiate from these progenitor populations. These include the spongiotrophoblast (SpT) and GlyT cells, which share a common EPC progenitor, and together with parietal trophoblast giant cells (P-TGCs), comprise the mature Jz. SpT cells function predominantly in the production of hormones, including members of the *Prl* (prolactin/placental lactogen-related) and *Psg* (pregnancy specific glycoprotein) gene families. The GlyT lineage is named for the abundant stores of glycogen that they accumulate and metabolise during gestation. In total, five TGC sub-types have been identified. Based upon lineage tracing, gene expression profiles and spatial localisation within the placenta (Fig 1), it is hypothesised that most of the TGCs also derive from the EPC (Simmons et al., 2007, Gasperowicz et al., 2013). TGCs associate with the maternal blood sinuses throughout the placenta and secrete hormones into the maternal circulation to modulate maternal physiology and placenta function. Sinusoidal-TGCs (S-TGCs) are located within the Lz, and together with a bilayer of syncytiotrophoblast (SynT-I and SynT-II) cells derived from the ExE, they create a trilaminar cellular arrangement that separates the maternal circulation from the extensively branched fetal vasculature (Adamson et al., 2002, Watson and Cross, 2005, Simmons et al., 2008a).

Based on lineage tracing experiments (Simmons and Cross, 2005), GlyT cells share a common *Tpbpa*$^{+}$ EPC progenitor with SpT cells and arise early in development. Periodic acid-Schiff (PAS) staining, which is a general staining method used to detect polysaccharides (e.g. glycogen) in addition to mucosubstances (e.g. glycoproteins and glycolipids), demonstrates small quantities of glycogen are stored by GlyT from ~E5.5 (Tesser et al., 2010), suggesting that GlyT are specified soon after implantation. An early lineage split of the GlyT and SpT populations is further suggested by expression of the GlyT-specific marker genes *Pcdh12* and

Aldh1a3 in a subset of EPC cells from ~E7.5 and ~E8.5, respectively (Rampon et al., 2005, Bouillot et al., 2005, Outhwaite et al., 2015). GlyT number expands nearly 300-fold between E12.5 and E16.5 before declining ~60% by E18.5 (Coan et al., 2006). This expansion coincides with a marked increase in PAS staining from E12.5 (Adamson et al., 2002), which distinguishes GlyT from the adjacent SpT cells (Fig 2A-C). By comparison, the SpT population increases less than four-fold during the same period, with a modest ~20% reduction by E18.5 (Coan et al., 2006). During this developmental timeframe, placental glycogen stores peak at ~E15.5 before declining by ~50% at E18.5 (Lopez et al., 1996) as the GlyT cell population diminishes. Even though both GlyT and SpT cells continue to express the Jz-marker gene *Tpbpa* in the mature placenta (Lescisin et al., 1988) (Fig 2D, E), other GlyT-specific markers have been described, including the gap-junction genes *Gjb3* (*Cx31*) and *Gjb5* (*Cx31.1*), which are localised to GlyT cells from ~E12.5 (Coan et al., 2006) and E13.5 (Zheng-Fischhofer et al., 2007), respectively, alongside persistent expression of *Pcdh12* (Fig 2F-H).

The putative role of placental glycogen

Studies in animal models have led to the hypothesis that placental glycogen stores provide a source of glucose to support fetal growth during late gestation (Coan et al., 2006). This hypothesis is supported by two observations; glycogen acts as the primary energy store in animals, and placental glycogen diminishes towards the end of pregnancy coincident with a period of rapid fetal growth. However, little experimental evidence currently exists to directly support such a function. The location of GlyT cells next to maternal blood sinuses may provide some indication as to the ultimate destination of the glucose released from placental glycogen stores. For instance, from ~E12.5 some GlyT invade the decidua where they localise in close proximity to maternal spiral arteries, and by E16.5 account for ~30% of the total GlyT population (Coan et al., 2006, Redline et al., 1993, Gasperowicz et al., 2013) (Fig 1). Based on this association, it is possible that migratory GlyT cells metabolise glycogen into glucose for transport into the maternal blood entering the placenta for eventual fetal uptake. Indeed, large lacunae form in the decidua by E17.5 that are presumed to result from the lysis of multiple GlyT in the vicinity (Bouillot et al., 2005). In contrast, populations of non-migratory GlyT cells cluster in close proximity to channels in the Jz that drain maternal blood from the placenta (Gasperowicz et al., 2013) (Fig 1). Whether these GlyT cells release glucose or other factors, such as hormones, into maternal blood to influence maternal physiology in

preparation for parturition and lactation (Napso et al., 2018) to indirectly support fetal growth and wellbeing is yet to be determined. Beyond location, the migratory and non-migratory GlyT populations are distinguishable by expression of distinct *Prl* genes: migratory GlyT express *Prl7b1* whilst non-migratory GlyT express *Prl6a1* (Simmons et al., 2008b). Further experiments are necessary to explore functional differences between these GlyT subtypes. Simultaneously, it is also possible that placental glycogen stores might provide energy directly to the placenta, since it is a highly metabolic organ that consumes a considerable proportion of the glucose delivered to it (Hay, 1995). Indeed, placental glycogen stores might fuel hormone production by the placenta (e.g. *Prl* and *Psg* gene family members), which increases dramatically towards term (Simmons et al., 2008b, McLellan et al., 2005).

How placental glycogen is metabolised is not well understood. Glucagon might play a role in stimulating the release of glycogen stores from GlyT (Coan et al., 2006). Yet, placental glycogen content is unaltered in mice deficient for the glucagon receptor (Ouhilal et al., 2012) indicating that an alternative pathway might be involved. However, only a small number of glucagon receptor mutants were examined at a single developmental time-point with no consideration of fetal sex. Fetal sex was shown to influence placental glycogen content, at least in the spiny mouse (O'Connell et al., 2013). Therefore, a role for glucagon in regulating glycogen metabolism cannot currently be excluded. Additional models are required to more fully understand the enzymes important for glycogen metabolism in the mouse placenta.

Common functionality of genes involved in GlyT development and function

Of the 47 mutant mouse lines that are known to exhibit GlyT phenotypes (with or without aberrant glycogen storage), 38 different genes are represented. As the list lengthens, common genetic pathways and gene classifications have emerged as important players in GlyT formation and function. The majority of these genes can be assigned to at least one of three functional groups (Table 1): imprinted and X-linked genes, cell signalling genes, and genes involved in transcriptional regulation.

Imprinted and X-linked genes

Imprinted genes represent an unusual class of autosomal gene that are characterised by complete or partial parental-allele-biased expression. This means that gene expression is derived wholly, or predominantly, from either the maternally inherited allele (i.e. maternally

expressed) or paternally inherited allele (i.e. paternally expressed) in one or more tissues. In excess of 100 imprinted genes have been reported in the mouse, many of which are known to regulate placental development and function (Tunster et al., 2013). A broad role for imprinted genes in regulating the GlyT lineage has been reviewed previously (Lefebvre, 2012), whereby four genes (*Ascl2*, *Cdkn1c*, *Igf2*, *Phlda2*) that reside within a ~1 Mb imprinted region on mouse distal chromosome 7 were discussed in detail. In addition to these genes, the paternally expressed *Dlk1*, *Peg3* and *Peg10* genes are also implicated in regulating the GlyT lineage and/or placental glycogen stores (Table 1), further emphasising the importance of imprinted genes in regulating placental glycogen stores.

A special class of imprinted genes is the X-chromosome-linked genes. To ensure similar gene dosage between males and females, one X chromosome in female cells is silenced. In extraembryonic lineages of female mouse conceptuses, the paternally inherited X-chromosome is preferentially silenced (Takagi and Sasaki, 1975). A role for X-lined genes in regulating placental glycogen storage is demonstrated by mice that inherited only a single paternally inherited X chromosome (XpO) and display and expansion of the GlyT population (He et al., 2017). In support of this hypothesis, GlyT phenotypes were reported to varying degrees in loss-of-function models of four X-linked genes (i.e. *Plac1*, *Ldoc1*, *Wdr1* and *Esx1*) (Table 1). The functional convergence of imprinted and X-linked genes in regulating placental glycogen storage is consistent with the established role of imprinted genes in modulating fetal nutrient supply across the placenta (Tunster et al., 2013, Angiolini et al., 2006) and with the purported function of placental glycogen stores in providing an energy source to support fetal growth during late gestation. With the majority of imprinted genes exhibiting placental expression in the mouse, it will be of interest to explore whether other imprinted genes are involved in regulating placental glycogen storage.

Cell-cell signalling

Studies of mutant mouse lines that report glycogen storage defects that arise secondary to SpT phenotypes implicate a role for SpT-derived signals in the regulation of placental glycogen metabolism. For instance, genetic knockout or over-expression of the maternally expressed imprinted gene *Phlda2* causes an expansion or reduction of the SpT population, respectively (Tunster et al., 2015, Tunster et al., 2010, Tunster et al., 2014). Unaltered expression of the GlyT marker genes *Pcdh12* and *Gjb3* suggested there was no overt effect on GlyT population

size in either model. However, placental glycogen content closely paralleled the SpT phenotype, with a reduced SpT population leading to diminished glycogen content and an expanded SpT population associated with increased glycogen storage (Tunster et al., 2015, Tunster et al., 2010, Tunster et al., 2014). These studies implicate cell-to-cell signalling between SpT and GlyT, though the specific pathways that mediate this effect are unclear. A clue might lie within the *Crim1* knockout model. *Crim1* encodes for a transmembrane protein that mediates signal transduction by binding growth factors (e.g. vascular endothelial growth factor A; VEGF-A) to the cell surface. While *Crim1* mRNA expression is limited to SpT cells, the GlyT population expands in the absence of CRIM1 function (Pennisi et al., 2012). Therefore, CRIM1 might regulate a SpT-derived factor that signals to GlyT to regulate their proliferation (Pennisi et al., 2012).

Further evidence for a role of SpT-derived signals in modulating placental glycogen stores is provided by a comparison of *Phlda2* null conceptuses and their wildtype littermates with strain-matched control litters. Loss of function of *Phlda2* results in expansion of the Jz (Frank et al., 2002), which is attributable to a disproportionate increase in the SpT population (Tunster et al., 2015). While there was no overt effect on GlyT cell number, an accumulation of glycogen in placentas from *Phlda2* null and their wild type littermates was apparent at E18.5. One explanation might be that the entire litter was exposed to an enhanced endocrine signalling environment caused by SpT expansion in *Phlda2* null placentas resulting in excessive placental glycogen storage that potentially deprives the fetuses of nutrients (Tunster et al., 2015). However, it is not possible to exclude a role for broader effects on maternal physiology in impairing fetal growth in this model.

We have identified a further fourteen genes that encode for specific components of cell signalling pathways that might help to establish the importance of cell-cell signalling in the regulation of placental glycogen content (Table 1). These genes include signalling molecules such as cytokines (*Csf2*), hormones (*Pthlh, Prl7d1*) and growth factors (*Pgf*), receptors (*Egfr*) and downstream effectors, such as kinases (*Akt1*, *Pik3ca*) and phosphatases (*Ptp4a2*). Some of these genes encode for key intermediates in a diverse range of signalling pathways, namely VEGF (e.g. *Crim1*), epidermal growth factor (EGF) (e.g. *Egfr*) and transforming growth factor (TGF) (e.g. *HtrA1*) pathways.

In particular, aberrant AKT signalling is implicated in GlyT phenotypes associated with genetic knockouts of *Ptp4a2*, *Tfap2c, Igf2* or *Phlda2* (Sharma et al., 2016, Takao et al., 2012,

Sferruzzi-Perri et al., 2017, Dong et al., 2012, Saxena et al., 2002, Frank et al., 1999). This, combined with the fact that loss of function of *Akt1* itself impairs placental glycogen storage (Yang et al., 2003), indicates that the AKT pathway is key to this process. AKT signalling is known to regulate specific cellular processes including growth, proliferation, metabolism and survival (Manning and Toker, 2017, Yu and Cui, 2016, Hermida et al., 2017). Specific to its role in glycogen storage, AKT also regulates glycogenesis through the inhibition of glycogen synthase kinase 3 (GSK3), a negative regulator of glycogen synthase (Cross et al., 1995, Diehl et al., 1998). Other models that show changes in the expression of *Igf2* or *Phlda2* (e.g. *Pcdh12* knockout (Rampon et al., 2008)) might also implicate AKT signalling defects in their GlyT phenotypes. While aberrant AKT signalling was reported in the placentas of human FGR infants (Yung et al., 2008), placental glycogen storage has not yet been investigated in the context of human FGR to date.

Ablation of *Akt1*, which encodes for protein kinase Bα (PKBα), causes fetal and placental growth restriction (Yang et al., 2003). A near complete loss of glycogen-containing trophoblast cells was reported (Yang et al., 2003), though this result was based solely on examination of PAS-stained placenta sections. The absence of PAS staining is more likely to indicate a failure to accumulate glycogen, rather than ablation of the GlyT lineage. Therefore, this model should be revisited for a more detailed analysis of the GlyT phenotype, including determination of spatiotemporal expression of markers such as *Pcdh12* (Bouillot et al., 2005), *Gjb3* (Coan et al., 2006), *Aldh1a3* (Outhwaite et al., 2015) and biochemical analysis of glycogen content (Lo et al., 1970). Regardless, the result is consistent with the role of AKT in regulating GSK3, and indirectly, glycogen synthase (Yang et al., 2003).

Upstream regulators of AKT signalling include PRL2 and IGF2, both of which have been implicated in placental development. The *Ptp4a2* gene encodes for the protein phosphatase PRL2 and when knocked out the placentas were small in size due to a substantially reduced Jz defined by a near complete loss of SpT with only a few non-migratory GlyT remaining (Dong et al., 2012). Whilst PAS staining was reduced, placental glycogen content was not directly quantified (Dong et al., 2012). *Ptp4a2*$^{-/-}$ placentas also exhibited reduced AKT phosphorylation (Dong et al., 2012) indicating that PRL2 is important for this process. The associated *Ptp4a2*$^{-/-}$ fetuses were growth restricted at E16.5. In fetal tissues, AKT and MAPK (mitogen activated protein kinase) pathways are activated via IGF2 signalling mediated primarily through binding IGF1R (insulin like growth factor 1 receptor) (Sferruzzi-Perri et al., 2017, Forbes and

Westwood, 2008). However, the absence of a placental phenotype in *Igf1r* null mice suggests that IGF2 acts through an alternative, unknown receptor in the placenta (Baker et al., 1993, Efstratiadis, 1998). Whether IGF2 activates AKT signalling in the placenta is unclear, and a detailed assessment of AKT signalling in the placenta of *Igf2* mutants is required. Additionally, further work is warranted to explore the broad role of AKT signalling in regulating placental glycogen stores by utilising mouse models with known GlyT phenotypes.

Transcriptional regulators

The GlyT population is highly dynamic from, with the number of GlyT cells increasing by nearly 300-fold between E12.5 and E16.5, and switching from an apparently glycogenic state to a glycogenolytic state (Lopez et al., 1996). These changes implicate major shifts in transcriptional regulation to modulate placental glycogen metabolism. A broad role for transcriptional regulators in modulating placental glycogen storage and/or the GlyT lineage has not been previously recognised. We identified eight genes encoding specific transcriptional regulators that are implicated in the development and/or function of the GlyT lineage (Table 1). One example is the endothelial-specific microRNA *miR-126a* (*miR-126*), which when ablated leads to global hypermethylation associated with dysregulated expression of placenta-specific genes (e.g. *Prl6a1*, *Pcdh12* and *Tpbpa*), and imprinted genes (e.g. *Igf2*, *Phlda2* and *Cdkn1c*) (Sharma et al., 2019). Genetic ablation of *miR-126a* results in Jz hyperplasia, attributable to a specific expansion of the GlyT population, without a change in SpT abundance. Consequently *miR-126a*$^{-/-}$ placentas accumulated ~50% more glycogen than controls and were associated with fetal growth restriction at E15.5 (Sharma et al., 2019). The specific targets of *miR-126a* are yet to be determined. Further work is required to fully elucidate the transcriptional networks involved in regulating the GlyT lineage and glycogen storage.

Using genetic mouse models to understand the function of placental glycogen

The association of altered placental glycogen storage with human pregnancy complications supports an important role for glycogen in achieving a successful pregnancy outcome (reviewed in Akison et al., 2017). The majority of mouse models with GlyT phenotypes show evidence of fetal growth restriction (FGR), which is consistent with the putative role of placental glycogen in supporting fetal growth (Tables 2 and 3). In contrast, the remaining

models showed GlyT phenotypes that were either associated with normal, enhanced or undetermined effects on fetal growth (Table 4), or embryonic lethality around mid-gestation (Table 5) that precluded a meaningful assessment of fetal growth.

In our evaluation of the literature, we observed that the extent to which GlyT phenotypes are characterised varies considerably between studies. Indeed, only a minority of these models was assessed using a direct biochemical determination of placental glycogen content. Instead, reduced glycogen storage or mobilisation was inferred from one or more of the following methods: reduced (or absent) PAS staining; reduced GlyT abundance as determined by histology and/or reduced genetic marker expression for GlyT cells; mislocalisation of GlyT within the Lz or a failure of GlyT cells to migrate to the decidua. As a result, there is a need for established criteria in describing GlyT phenotypes and glycogen storage and metabolic defects. We identified four key parameters for characterising GlyT phenotypes: 1. GlyT lineage specification and differentiation by assessing lineage marker expression, 2. GlyT cell number, 3. GlyT localisation and degree of migration into the decidua, and 4. Quantification of total placental glycogen content using a biochemical assay (Lo et al., 1970). A collective evaluation of these genetic mutants using these criteria will help to separate GlyT developmental defects from metabolic/storage defects and improve our understanding of how placental glycogen stores support growth.

Reduced placental glycogen content is frequently associated with fetal growth restriction

Several mouse models that demonstrate a glycogen storage deficit are also associated with FGR (Table 2). It is well known that IGF2 is an important regulator of fetal and placental growth (DeChiara et al., 1990, Constância et al., 2005). *Igf2* mRNA is highly expressed in the GlyT lineage (Georgiades et al., 2002, Redline et al., 1993, Coan et al., 2006) and constitutive deletion of *Igf2* results in reduced GlyT abundance and placental glycogen content (Lopez et al., 1996). Deletion of the placenta-specific *Igf2* transcript (*Igf2P0*) results in a similar reduction of GlyT cell number (Sferruzzi-Perri et al., 2011), even though the *Igf2P0* transcript accounts for only 10% of total placental *Igf2* (Moore et al., 1997). Whilst constitutive deletion of *Igf2* results in a 50% reduction in fetal weight (DeChiara et al., 1990), *Igf2P0* mutants are 25% lighter than controls (Constância et al., 2005), at least partially attributing FGR to a placental defect. However, the contribution of placental glycogen is unclear as glycogen content was not directly assessed in the *Igf2P0* mutants.

Human placentas exhibiting elevated expression of *PHLDA2* are frequently linked with FGR (reviewed in Jensen et al., 2014), although placental glycogen stores have not been investigated in these placentas. Analysis of a mouse model with over-expression of *Phlda2* demonstrates that placental glycogen content is reduced by 50% of controls. However, the effect on glycogen storage is likely secondary to a reduction in the *Prl8a8*$^{+}$ SpT population since there was no overt effect on expression of the GlyT markers *Pcdh12* or *Gjb3* (Tunster et al., 2010, Tunster et al., 2014, Tunster et al., 2015). The effect on fetal growth in the *Phlda2* over-expression model is complex and dependent upon the genetic background of the mouse, even though similar placental defects are apparent between strains. For instance, fetal growth was asymmetrically restricted when on the 129S2/SvHsd background (Salas et al., 2004, Tunster et al., 2010), but was unaffected on the C57BL/6 background (Tunster et al., 2014). Since genetically wildtype placentas on the C57BL/6 background naturally accumulate more than twice the glycogen content of 129S2/SvHsd placentas (Tunster et al., 2012), fetal growth might be protected on the C57BL/6 background.

Even though GlyT are important for fetal growth, these cells are not essential to fetal survival. GlyT cells were absent in mutant mouse lines whereby the expression of *Ascl2* was at ~50% of endogenous levels (*Ascl2*$^{LacZ/Del7AI}$ or *Del*^{7AI}/+ mutants). Placentas either demonstrated a lack of PAS stain and/or *Pcdh12* expression at E15.5. The associated fetuses survived to term, albeit exhibiting growth restriction of 15-20% (Lefebvre et al., 2009, Oh-McGinnis et al., 2011, Bogutz et al., 2018). In contrast, *Ascl2* null placentas lack the EPC progenitor cells that give rise to the SpT and GlyT resulting in embryonic lethality at mid-gestation (Guillemot et al., 1994). Therefore, *Ascl2* is likely required for the formation of GlyT cells and might also be associated with glycogen storage or metabolism.

Beyond GlyT differentiation, several mouse strains demonstrate abnormal localisation of GlyT (Table 2). Normally, GlyT are present in the Jz with a subpopulation of GlyT migrating into the decidua (Coan et al., 2006). *Ldoc1*, *HtrA1* and *Tfap2c* single knockout mutations lead to placentas with GlyT that have mislocalised to the Lz (Hasan et al., 2015, Naruse et al., 2014, Kaiser et al., 2015). The Jz naturally interdigitates with the Lz (Soares and Hunt, 2014), with the extent to which depends upon gestational stage and genetic background (Tunster et al., 2012). Therefore, it is important to be cautious when histologically characterising clusters of GlyT located in the Lz. Serial sections of placenta can be assessed to differentiate a GlyT cluster from a Jz finger protruding in to the Lz. Alternatively, failure of GlyT to migrate into

the decidua, as in *Ptp4a2*$^{-/-}$ or *Phlda2* over-expression mice (Dong et al., 2012, Tunster et al., 2010), might affect maternal-fetal interactions. Whether defective GlyT migration in these models results from altered cell-signalling pathways associated with adhesion and cytoskeleton, or an inappropriate response to chemotactic signals remains to be determined. However, appropriate localisation of GlyT appears to be important for normal fetal growth.

Increased placental glycogen storage is also associated with impaired fetal growth

Unexpectedly, FGR can also occur in the context of increased placental glycogen storage. For example, when the number of GlyT cells exceeded 35% of the Jz as in interspecific mouse hybrids, fetal growth was inversely correlated with GlyT abundance (Kurz et al., 1999). Additionally, several genetic mouse lines exist that demonstrate increased placental glycogen storage associated with FGR (Table 3). One potential explanation for these counterintuitive findings is that the mutant placentas are able to store glycogen but cannot effectively mobilise and release the glycogen. Further work is necessary to elucidate the mechanism, with a specific focus on whether glycogenesis is increased, leading to enhanced glycogen storage, whether glycogenolysis is impaired, leading to failed glucose release, or whether transport mechanisms are defective. Alternatively, FGR in models associated with increased placental glycogen storage might be caused by a mechanism independent of the GlyT or placental glycogen. Of note, some models (e.g. *Ascl2*-Tg, *Pcdh12*$^{-/-}$ and *Plac1*$^{-/-}$) with increased glycogen storage also display mislocalisation of GlyT clusters within the Lz (Tunster et al., 2016, Rampon et al., 2008, Jackman et al., 2012). This result is consistent with a role for the normal microenvironment of the Jz in modulating the mobilisation of glycogen stores by the GlyT.

Enhanced fetal growth might be independent of a GlyT phenotype

It is possible for enhanced fetal growth to associate with aberrant placental glycogen storage, as occurs in *Cdkn1c*$^{-/+}$ and *H19*$^{-/-}$ mice (Tunster et al., 2011, Esquiliano et al., 2009) (Table 4). However, it is unlikely that these placental phenotypes directly cause fetal overgrowth since these genes exert an intrinsic effect on fetal growth. For instance, conditional over-expression of *Cdkn1c* in a subset of fetal, but not placental, tissues restricts fetal growth (Andrews et al., 2007, John et al., 2001). In contrast, whilst *Cdkn1c* knockout mice exhibit fetal overgrowth and placentomegaly at E15.5 and E18.5, fetal weights are normalised at birth (Tunster et al., 2011). This failure to sustain fetal over-growth may be attributable to the manifestation of

severe defects in *Cdkn1c*$^{-/+}$ placentas including substantially diminished glycogen stores (Tunster et al., 2011), although GlyT abundance is unaffected (Takahashi et al., 2000). Similarly, ablation of *H19* drives over-expression of the growth-promoting gene *Igf2*, resulting in neonates that are 30% heavier than their littermates (Leighton et al., 1995), and placentas with increased GlyT number and placental glycogen content (Esquiliano et al., 2009). However, since the *H19*-driven fetal over-growth phenotype persists to adulthood, the cause is likely independent of increased placental glycogen stores.

GlyT phenotypes associated with early embryonic lethality

Several genetic mouse models display a GlyT phenotype and result in embryonic lethality at or around mid-gestation (Table 5). This outcome occurs before GlyT cells mature and begin accumulating glycogen stores in earnest. Early fetal demise prevents a meaningful assessment of the effect of placental glycogen stores on fetal growth in this context. It is clear that embryonic lethality is attributable to a placental defect in the case of *Ascl2* and *Peg10* knockouts, since lethality was rescued using a tetraploid aggregation approach (Guillemot et al., 1995, Guillemot et al., 1994, Ono et al., 2006). This method generates a chimeric conceptus in which a mutant fetus is supported by a placenta containing wild-type tetraploid cells (Nagy et al., 1990, Tarkowski et al., 1977). In this context, rescue of embryonic lethality indicates a placental cause. Embryonic lethality in the remaining models coincides with the appearance of congenital malformations (Table 5). A conditional knockout approach is required to elucidate the role of these genes in later stage placentas, including in GlyT development and function. A recent study demonstrated that defects in placental development and/or function might contribute to the formation of congenital malformations in the fetus, particularly those affecting heart, brain and vascular development (Perez-Garcia et al., 2018). The extent to which GlyT and placental glycogen stores play a role in this phenomenon is yet-to-be determined.

Limitations

Whilst the studies presented here broadly support a role for placental glycogen in regulating fetal growth, a major limitation to understanding the true cause-effect relationship is that the Lz structure is also disrupted in many of these models (Tables 2-5). The Lz facilitates the exchange of nutrients, gases and waste products between maternal and fetal circulations

(Watson and Cross, 2005). Reduced branching morphogenesis in the Lz decreases the surface area for nutrient transport, compromising placental function (Cross et al., 2006). Consequently, it is difficult to directly attribute the adverse fetal growth outcomes specifically to aberrant placental glycogen storage for most genes. One model whereby fetal growth and placental glycogen content might show a direct link is a hypomorphic *Egfr* mutation. These mutant mice have placentas with a GlyT-specific phenotype characterised by reduced PAS staining and diminished GlyT marker expression but no other placental defect. Associated fetuses were growth restricted in a background-specific manner, with *Egfr* hypomorphs that restricted on the 129Sv and BTBR/J backgrounds but not the C57BL/6J background (Dackor et al., 2009). Crucially, whilst PAS staining of *Egfr* hypomorphic placentas was reduced on the 129Sv and BTBR/J backgrounds, abundant PAS staining was evident on the C57BL/6 genetic background (Dackor et al., 2009). This emphasises a potential relationship between glycogen storage and fetal growth.

The ability to ascertain the mechanism driving aberrant glycogen storage from existing studies is difficult given that GlyT abundance and total placental glycogen content are not always assessed together. Therefore, it is unclear whether an early defect in GlyT progenitor maintenance or differentiation leading to a reduction in mature GlyT cells is the main cause of glycogen storage defects. This appears to be the case for at least eleven mutant mouse lines where both parameters have been assessed (Table 1). The majority of studies have assessed only a single parameter: GlyT abundance or placental glycogen content. This will affect the conclusions drawn by each study and not necessarily reflect whether the mechanism is developmental or metabolic in origin. The only known model whereby placental glycogen stores are independent of GlyT differentiation and abundance is the *Cdkn1c* null line (Tunster et al., 2011, Takahashi et al., 2000). From this model, we might better understand how glycogen metabolism occurs in the placenta and how fetal growth responds.

Future directions

Considerable work is required to fully elucidate the normal physiological role of placental glycogen stores in humans given that we are only beginning to understand its role during pregnancy. To improve our understanding, a systematic analysis of the GlyT population is required in known mouse models with placenta phenotypes and as part of the routine assessment of new mouse placenta phenotypes. We have proposed four key parameters to

assess the GlyT lineage to better understand their development and function: 1) Quantification of GlyT cell number as determined by nuclear counts on histological sections; 2) Assessment of GlyT specific marker gene expression (e.g. *Pcdh12*, *Aldh1a3*, *Gjb3*) either by *in situ* hybridisation on histological sections (Fig 2) or by qPCR analysis at multiple stages of development. This should occur in concert with assessment of other trophoblast lineage marker expression; 3) Analysis of the localisation of GlyT cells by undergoing *in situ* hybridisation for the migratory (*Prl7b1*) and non-migratory (*Prl6a1*) markers. This will indicate the migratory capacity of these cells and whether placental structure is affected; and 4) Exploring placental glycogen content by performing PAS-staining of histological sections (Fig 2) alongside quantification of total placental glycogen content using a biochemical assay (Lo et al., 1970). Altogether, these criteria will help to direct subsequent phenotype evaluation, and ultimately identify further molecules and pathways important for GlyT formation and function, and for the regulation of placental glycogen stores.

Given the highly dynamic nature of the GlyT cells over the course of pregnancy, it is important to assess placental glycogen-related phenotypes (and arguably other placental phenotypes) at multiple gestational stages. Characterisation of a single developmental stage might misrepresent the full extent of the phenotype. One model that exemplifies this issue is the *Pthlh* knockout mouse (Duval et al., 2017), whereby placental glycogen content was reduced at E12.5 and E14.5. By E16.5, glycogen had normalised to control levels. However, glycogen content was elevated at E18.5 relative to controls, failing to exhibit the anticipated decline towards term that is normally attributed to the mobilisation of glycogen stores in late gestation. Coinciding with the glycogen phenotype, fetal growth was restricted from E16.5 (Duval et al., 2017). Although *Pthlh*-null mice exhibit severe skeletal abnormalities and die soon after birth (Karaplis et al., 1994, Bond et al., 2008), the late-onset FGR observed in this model implicates a causal role for placental dysfunction. However, it is unclear whether FGR can be attributed to impaired glycogen storage during mid-gestation or the apparent failure to mobilise placental glycogen stores at term. Assessing phenotypes at multiple stages of development will potentially allow for better separation of developmental defects from functional defects.

The regulation of glycogenesis and glycogenolysis pathway activity by GlyT will provide further insight into placental glycogen metabolism. Central to glucose release from a cell is the enzyme glucose 6-phosphatase, which is responsible for generating free glucose by

hydrolysing the polar phosphate group from glucose 6-phosphate and allowing its transport out of the cell (van Schaftingen and Gerin, 2002). The absence of glucose 6-phosphatase activity in GlyT cells would prevent the release of glucose for consumption by the fetus, mother or trophoblast cells. While expression of glucose 6-phosphatase has not been investigated in the mouse placenta to date, its activity in the human placenta is attributed to the *G6PC3* isoform (Guionie et al., 2003). This isoform is distinct from the liver-specific *G6PC1* isoform, and is expressed in the placenta from at least week 28 of human pregnancy (Matsubara et al., 1999), and may be associated with the production of glucose in the placenta at term (Prendergast et al., 1999). However, the ultimate destination of glucose derived from placental glycogen stores remains unclear. It is possible that it is directed for fetal use and/or placental consumption. Signals from the fetus that release placental glycogen stores have not been identified.

It is also possible that placental glycogen stores support maternal physiology. The localisation of non-migratory GlyT around channels that drain maternal blood from the placenta suggests that some of the glucose released from placental glycogen stores may first be available for uptake by the mother. GlyT express several *Prl* genes and the localisation of non-migratory GlyT suggest the encoded hormones may be released into maternal blood. Whilst placental hormones are known to adapt maternal physiology to pregnancy, there is currently no evidence that the glucose metabolised from placental glycogen stores acts to the benefit of maternal physiology. Future studies should assess the effects of aberrant placental glycogen storage on maternal physiology. Indeed, genetic perturbations of several genes that regulate the GlyT lineage also have effects on maternal behaviour, including *Peg3* (Li et al., 1999, McNamara et al., 2018) and *Phlda2* (Creeth et al., 2018). Little is known about changes to maternal physiology in these models. *Dlk1*$^{+/-}$ mice are the only model in which maternal physiology was characterised in association with a placental GlyT phenotype (Cleaton et al., 2016, Appelbe et al., 2013). However, the effects on maternal physiology are more likely attributable to the loss of circulating DLK1 (also known as fetal antigen-1; FA1), rather than a consequence of altered placental glycogen metabolism.

Conversely, there is evidence that maternal physiology can regulate placental glycogen stores. A loss of function allele of *Pik3ca*, which encodes for the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), showed a maternal zygotic effect resulting in diminished glycogen stores (Sferruzzi-Perri et al., 2016), although the underlying mechanism is not well

understood. While manipulation of maternal diet modulates fetal growth in mouse models (Jones et al., 2009, Zhang et al., 2005, Zhang et al., 2009), only a few studies have investigated the effect of maternal diet upon placental glycogen storage. Both calorie and protein restriction in dams impair placental glycogen storage (Gonzalez et al., 2016, Coan et al., 2010, Sferruzzi-Perri et al., 2011), presumably as a consequence of diminished nutrient availability. A high sugar, high fat (HSHF) maternal diet results in FGR at E15.5, a phenotype that is normalised by E18.5 (Sferruzzi-Perri et al., 2013), potentially due to increased utilisation of glycogen to support accelerated fetal growth during late gestation. Further work is necessary to explore the relationship between maternal diet, placental glycogen metabolism and fetal growth.

Attempts to infer the function of placental glycogen in models where the GlyT lineage is ablated or diminished might be confounded by additional functions of GlyT cells, which perform at least two additional functions to glycogen storage. Firstly, GlyT cells contribute to placental endocrine function as evidenced by their expression of a subset of the *Prl* gene cluster (Simmons et al., 2008b). Secondly, GlyT expression of ALDH1A3, an enzyme that oxidises retinal to retinoic acid, implies that GlyT might be a source of retinoic acid, which is an important regulator of trophoblast differentiation (Outhwaite et al., 2015). Therefore, alterations in the GlyT population might have profound effects on placental structure and function independent of glycogen storage.

Overall, better characterisation of GlyT phenotypes in mice will allow us to evaluate the glycogenesis and glycogenolysis pathways involved in storing and metabolising glycogen in normal pregnancies and pathological contexts. More sophisticated approaches to constitutive gene knockout models will be necessary to elucidate the specific physiological role(s) of placental glycogen. These analyses should include single-cell sequencing approaches to better understand genetic and metabolic pathways within GlyT cells together with a more holistic approach that relates placental structure and glycogen metabolism to fetal growth and development, and to maternal physiology. Only then will the normal function and relevance of aberrant placental glycogen stores to human pregnancy complications, such as PE and GDM, be clarified.

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Author contributions

SJT conceived the study. SJT, EDW, ALF and GJB wrote and edited the manuscript. All authors have read and approved the final version of this manuscript.

Declaration of interest

EDW is an associate editor at Reproduction.

Figure Legends

Figure 1: Structure and trophoblast lineages of the mature mouse placenta: The mature mouse placenta comprises three structurally and functionally distinct layers: the maternal decidua (Dec), junctional zone (Jz) and labyrinth zone (Lz). The junctional zone predominantly comprises the spongiotrophoblast (SpT) and non-migratory glycogen trophoblast (GlyT) cells. A proportion of GlyT migrate to the maternal decidua where they associate with maternal spiral arteries bringing maternal blood to the placenta. Five trophoblast giant cell sub-types have been described based on localisation on gene expression patterns: the parietal TGCs (P-TGCs) form a discontinuous cell layer at the boundary between the Jz and maternal decidua; spiral artery-associated TGCs (SpA-TGCs) line maternal spiral arteries through the decidua; canal-TGCs (C-TGCs) line maternal blood canals through the Jz and Lz; sinusoidal-TGCs (S-TGCs) replace the endothelial layer of maternal blood sinuses within the Lz; channel-TGCs (Ch-TGCs) surround the channels that traverse the Jz draining maternal blood from the placenta. Together with the S-TGCs, two layers of syncytiotrophoblast form the trilaminar structure of the murine labyrinth.

Figure 2: Histological methods for assessing glycogen trophoblast cells in the mouse placenta at E14.5.

Histological sections of wild type (C57BL/6J) mouse placentas at E14.5 stained using (A-C) Periodic acid-Schiff (PAS) stain (dark pink) that labels glycogen, glycoproteins and glycolipids, (D-E) *Tpbpa* RNA via an *in situ* hybridisation probe (dark purple) that stains cells derived from the ectoplacental cone (e.g. spongiotrophoblast (SpT) and glycogen trophoblast (GlyT)), and (F-H) *Pcdh12* RNA via an *in situ* hybridisation probe (dark purple) that stains GlyT. Nuclei are stained using (A-C) haematoxylin (blue) or (D-H) nuclear fast red (pink). Dec, maternal decidua; Jz, junctional zone; Lz, labyrinth zone; YS, yolk sac; UC, umbilicus (black arrow); SpA, spiral artery; m, maternal blood sinusoid; mGlyT, migratory GlyT (blue arrows). Dotted lines separate major placenta layers or trophoblast cell populations. Boxes represent regions shown in higher magnification to the right. Scale bars: A, D, F = 1 mm; B, E = 250 μm; C = 50 μm; G, H = 100 μm

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Table 1: Classification and expression of genes implicated in regulating GlyT differentiation and/or function

Symbol	Gene name	Function	Spatial expression	GlyT differentiation†	Glycogen storage††	References
Imprinted and X-linked genes						
Ascl2	*Achaete-scute complex homolog 2*	Maternally expressed basic helix-loop helix transcription factor	Highly expressed in both EPC and ExE during early development. Expression declines towards term	✓	✓	(Guillemot et al., 1994, Rossant et al., 1998)
Cdkn1c	*Cyclin-dependent kinase inhibitor 1C*	Maternally expressed cyclin dependent kinase inhibitor	Widely expressed with high expression in GlyT	×	✓	(Georgiades et al., 2002, Coan et al., 2006, Matsuoka et al., 1995)
Dlk1	*Delta-like homolog 1*	Paternally expressed transmembrane glycoprotein. Encodes the precursor to fetal antigen 1 (FA1) that functions as a Notch antagonist.	Expressed in endothelial cells lining the fetal vasculature of the Lz. Not expressed in any trophoblast lineage.	✓	n/a	(Yevtodiyenko and Schmidt, 2006, Baladron et al., 2005, Laborda, 2000)
Igf2	*Insulin-like growth factor 2*	Paternally expressed insulin-like growth factor 2	Highly expressed in Jz and Lz between E9.5 and E12.5. Expression subsequently declines dramatically in the Lz and SpT but remains highly expressed in GlyT.	✓	✓	(Redline et al., 1993, Carter et al., 2006)
Peg3	*Paternally expressed gene 3*	Paternally expressed zinc finger transcriptional repressor	Widely expressed in the developing placenta	✓	✓	(Relaix et al., 1996)
Peg10	*Paternally expressed gene 10*	Paternally expressed retrotransposon derived gene	Widely expressed in all trophoblast lineages	✓	n/a	(Ono et al., 2006)
Phlda2	*Pleckstrin homology-like domain family A member 2*	Maternally expressed pleckstrin-homology domain protein that inhibits the AKT pathway by competing for PIP targets	Highly expressed in the EPC and ExE. Restricted to the chorionic plate and syncytiotrophoblast layers of the Lz by E10.5. Expression declines dramatically from E12.5. Very few PHLDA2-positive cells remain at E14.5	n/a	✓	(Frank et al., 1999, Frank et al., 2002, Saxena et al., 2002)
Esx1	*Extraembryonic, spermatogenesis, homeobox 1*	X-linked paired-like homeobox domain protein	Expressed in the EPC and chorion before becoming restricted to the Lz by mid-gestation	✓	n/a	(Li et al., 1997, Li and Behringer, 1998)

Plac1	*Placental specific protein 1*	X-linked putative signal peptide	Placenta specific expression from early to mid-gestation. EPC and TGCs at E7.5; P-TGCs, Jz and Lz, by E11.5; restricted to P-TGCs by E14.5	n/a	✓	(Cocchia et al., 2000)
Ldoc1	*Regulator of NFKB signalling*	X-linked gene derived from long terminal repeat retrotransposon	Highly expressed in TGCs, EPC and ExE at E8.5. Restricted to non-migratory GlyT by E15.5 and absent by E18.5.	×	n/a	(Naruse et al., 2014)
Wdr13	*WD repeat domain 13*	X-linked member of WD-repeat protein family	Expressed predominantly in the Lz. Lower expression in the SpT and decidua. Not expressed in GlyT	n/a	✓	(Singh et al., 2015)
Cell-cell signalling						
Akt1	*Thymoma viral proto-oncogene 1*	Encodes protein kinase Bα (PKBα), a downstream effector of the phosphatidylinositol 3-kinase (PI3K) signalling pathway	Widely expressed in all trophoblast lineages and fetal endothelium	n/a	✓	(Yang et al., 2003)
Crim1	*Cysteine rich transmembrane BMP regulator 1*	Transmembrane protein that binds growth factors such as VEGF-A to the cell surface	Primarily expressed in SpT. Low expression in P-, C- and syncytiotrophoblast	n/a	×	(Pennisi et al., 2007, Pennisi et al., 2012, Wilkinson et al., 2007)
Csf2	*Colony stimulating factor 2 (granulocyte-macrophage)*	Encodes the cytokine Granulocyte-macrophage colony-stimulating factor 2	Expressed in the decidua and Jz	✓	n/a	(Kanzaki et al., 1991, Robertson et al., 1999)
Egfr	*Epidermal growth factor receptor*	Encodes epidermal growth factor receptor (EGFR)	Expressed in Jz and Lz at E18.5	✓	✓	(Dackor et al., 2007)
HtrA1	*HtrA serine peptidase 1*	Serine protease that functions as an antagonist of TGF-β signalling	Expressed in P-TGCs at E7.5. Expression is barely detectable by E13.5.	×	×	(Nie et al., 2005, Hasan et al., 2015, Oka et al., 2004)
Hectd1	*HECT domain E3 ubiquitin protein ligase 1*	Ubiquitin ligase that functions as a negative regulator of Wnt signalling	Widely expressed in all regions of the placenta between E7.5 and E13.5.	✓	✓	(Sarkar et al., 2014, Tran et al., 2013)
Igfbp1	*Insulin-like growth factor binding protein 1*	Carrier protein for insulin-like growth factors	Strongly expressed in the yolk sac endoderm but not expressed in trophoblast lineages	✓	n/a	(Carter et al., 2006, Hwa et al., 1999)
Pcdh12	*Protocadherin 12*	Member of the protocadherin subgroup of the cadherin family of transmembrane	Expressed exclusively in GlyT lineage throughout gestation	n/a	✓	(Rampon et al., 2005)

		calcium-dependent cell-adhesion proteins				
Pgf	*Placental growth factor*	Encodes the pro-angiogenic Placental Growth Factor (PlGF)	Widely expressed in the placenta at E10.5	✓	✓	(Achen et al., 1997, Tayade et al., 2007)
Pik3ca	*Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha*	Encodes the ubiquitously expressed PI3-kinase catalytic subunit p110α	Ubiquitously expressed	n/a	✓	(Foukas et al., 2006)
Prl7d1	*Prolactin family 7, subfamily d, member 1*	Member of the prolactin/placental lactogen (*Prl/Pl*) gene family	Widely expressed in all trophoblast sub-types; highest expression in GlyT	✓	✓	(Simmons et al., 2008)
Ptp4a2	*Protein tyrosine phosphatase 4a2*	Protein phosphatase	Widely expressed in all trophoblast lineages	✓	✓	(Dong et al., 2012)
Pthlh	*Parathyroid hormone-like peptide*	Encodes the constitutively expressed parathyroid hormone-related peptide (PTHrP)	Constitutively expressed	n/a	✓	(Duval et al., 2017, Wysolmerski and Stewart, 1998)
sFlt-1	*Soluble fms related receptor tyrosine kinase 1*	An anti-angiogenic factor that sequesters placental growth factor (PGF) and vascular endothelial growth factor (VEGF)	Expressed in the EPC and later the Jz	✓	n/a	(Breier et al., 1995, Kendall and Thomas, 1993, Levine et al., 2006)
Transcriptional regulators						
Arnt	*Aryl hydrocarbon receptor nuclear translocator*	Encodes the HIF1β subunit of the heterodimeric transcription factor hypoxia inducible factor (HIF)	Constitutively expressed	✓	n/a	(Adelman et al., 2000)
Bahd1	*Bromo adjacent homology domain containing 1*	Chromatin modifier that mediates heterochromatin-associated gene silencing. Implicated in the silencing of *IGF2* in humans	Not reported	n/a	✓	(Bierne et al., 2009)
Cited2	*Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2*	Transcriptional co-factor	Expressed in all trophoblast lineages.	✓	n/a	(Dunwoodie et al., 1998, Withington et al., 2006)
Hif1a	*Hypoxia inducible factor 1, alpha subunit*	Encodes the HIF1α subunit of the heterodimeric transcription factor hypoxia inducible factor (HIF)	Constitutively expressed but HIF1A protein is degraded in normoxia.	✓	n/a	(Cowden Dahl et al., 2005, Kozak et al., 1997)
miR-126a	*microRNA 126a*	Endothelial-specific microRNA	Widely expressed in fetal endothelium of Lz, syncytiotrophoblast, P-TGC, SpT and GlyT.	✓	✓	(Sharma et al., 2019, Wang et al., 2008)
Prdm1	*PR domain containing*	Zinc-finger transcriptional repressor	Expressed in GlyT progenitors at	n/a	n/a	(Mould et al., 2012)

	1, with ZNF domain		E7.5. Highly expressed in SpA-TGCs.			
Tfap2c	*Transcription factor AP-2, gamma*	Transcription Factor AP-2 Gamma	Expressed in the trophectoderm at E4.5. Restricted to Jz and TGCs by E11.5.	✓	✓	(Kuckenberg et al., 2010)
Sp1 & Sp3	*Trans-acting transcription factor 1 & 3*	Zinc-finger transcription factors	Ubiquitously expressed	n/a	n/a	(Kruger et al., 2007)
Miscellaneous						
Cts7	*Cathepsin 7*	Placenta-specific papain-like cysteine cathepsin protease that functions in lysosomal proteolysis	Highly expressed in TGCs at E7.5; expression declines and is restricted to the Lz by E15.5	✓	n/a	(Hemberger et al., 2000, Deussing et al., 2002)
Fbxl12	*F-box and leucine-rich repeat protein 12*	Member of the F-box family of proteins, which function as the substrate recognition component in E3 ubiquitin ligase complexes	Jz specific at E15.5	n/a	✓	(Nishiyama et al., 2015, Zheng et al., 2016, Nakayama and Nakayama, 2006)
Pdcd5	*Programmed cell death 5*	Promoter of both apoptotic and the non-apoptotic programmed cell death pathway (paraptosis)	Not reported	✓	✓	(Wang et al., 2004, Liu et al., 1999)
Tex19.1	*Testis expressed gene 19.1*	Mammalian-specific hypomethylation-sensitive genome-defence gene	Widely expressed in the EPC and Jz; some patchy expression in Lz	✓	n/a	(Celebi et al., 2012, Reichmann et al., 2013)

† Changes in GlyT abundance or maturation implied an effect in GlyT differentiation. †† Altered regulation of glycogen stores is suggested by an effect on total placental glycogen content (see Tables 2-5 for details and references); n/a, data not available. E, embryonic day; EPC, ectoplacental cone; ExE, extraembryonic ectoderm; GlyT, glycogen trophoblast cells; Jz, junctional zone; Lz, labyrinth zone; P, postnatal day; PAS, Periodic Acid Schiff stain; SynT-II, syncytiotrophoblast cell layer II; TGC, trophoblast giant cell; TGC subtypes: C-TGC, canal TGC; P-TGC, parietal TGC; S-TGC, sinusoidal TGC; SpA-TGC, spiral artery associated TGC.

Table 2: Genetic mouse models with fetal growth restriction and placentas with reduced glycogen content and/or GlyT phenotypes

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content	GlyT number	GlyT markers	GlyT localisation	Lz phenotype	References
Akt$^{-/-}$	Knockout	↓ (E14.5, E16.5)	↓↓ (E14.5, E16.5)	↓ PAS	n/a	n/a	n/a	Reduced Lz size	(Yang et al., 2003)
Del^{7AI}*/+*	*Ascl2* expressed reduced by 40%	↓ (P0)	↓↓ (E15.5)	n/a	↓↓↓↓ (E15.5)	↓ *Tpbpa* ↓↓↓↓ *Pcdh12*	n/a	Disorganised structure	(Lefebvre et al., 2009, Oh-McGinnis et al., 2011)
Ascl2$^{LacZ/Del7AI}$	*Ascl2* expressed reduced by 60%	↓↓ (E15.5)	↓↓ (E15.5)	↓↓↓↓ PAS	↓↓↓↓ (E15.5)	↓ *Tpbpa* ↓↓↓↓ *Pcdh12*	n/a	Disorganised structure	(Bogutz et al., 2018)
Bahd1$^{-/-}$	Knockout	↓↓ (E18.5)	n/a (↓ circumference and area at E18.5)	↓ PAS	n/a	n/a	n/a	Reduced Lz size	(Lakisic et al., 2016)
Csf2$^{-/-}$	Mixed 129/Sv x C57BL/6 background	↓ (E16.5)	Normal (E16.5)	n/a	↓↓	n/a	n/a	Reduced Lz size	(Kanzaki et al., 1991, Robertson et al., 1999)
Dlk1$^{+/-}$	Knockout*	↓ (E18.5, P7)	Normal (E12.5, E14.5, E16.5)	n/a	↑ progenitors ↓ mature GlyT	n/a	n/a	Reduced Lz size; vascularisation defect	(Yevtodiyenko and Schmidt, 2006, Appelbe et al., 2013, Cleaton et al., 2016)
Egfr$^{-/-}$	Knockout	↓ (from E13.5)	↓ (from E11.5)	n/a	n/a	↓ *Tpbpa* (E13.5)	n/a	n/a	(Sibilia and Wagner, 1995)
Egfr^{wa2}	*Egfr* hypomorph	↓ (E18.5) (Effects are genetic background dependent)	↓↓ (variable, E15.5, E18.5)	↓ PAS	n/a	↓ *Tpbpa* ↓ *Pcdh12*	n/a	Increased expression of *Gcm1, Dlx3, Tcfeb*	(Dackor et al., 2009)
Fbxl12$^{-/-}$	Knockout	↓ (E17.5)	↓↓↓ (E17.5)	↓ PAS	n/a	↓ *Tpbpa* ↓ *Pcdh12* ↓ *Gjb3*	n/a	Vascularisation defect	(Nishiyama et al., 2015)
hIGFBP1-Tg	Over-expression of human *IGFBP1*	↓ (E11.5) Normal (E14.5)	↑ (E11.5)	n/a	↓ GlyT:SpT ratio (in	n/a	n/a	Increased Lz size	(Crossey et al., 2002)

					females)				
HtrA1$^{-/-}$	Knockout	↓ (E14.5)	↓ (E14.5)	n/a	n/a	↓ *Tpbpa*	Mislocalisation of *Tpbpa*$^{+}$; PAS^{+} cells within Lz	n/a	(Nie et al., 2005, Hasan et al., 2015)
Igf2$^{-/-}$ or *Igf2*$^{+/-}$	Fetal and placental knockout*	↓ (E16.0)	↓↓↓ (E15.5) ↓↓ (E18.5)	↓↓↓ glycogen (E15.5, E18.5)	↓↓↓ (E15.5, E18.5)	n/a	n/a	n/a	(Redline et al., 1993, Carter et al., 2006, Lopez et al., 1996, DeChiara et al., 1990)
Igf2P0	Loss of placental-specific *Igf2* transcripts*	↓ (from E16.5)	↓ (E13.5) ↓↓ (E15.5, E18.5)	n/a	↓↓ (E15.5)	n/a	n/a	Reduced Lz size, Increased trophoblast barrier thickness, Reduced diffusional capacity	(Constância et al., 2005, Sferruzzi-Perri et al., 2011, Sibley et al., 2004)
LV-hsFLT-1	Over-expression of human *sFLT-1*	↓ (E18.5)	↓ (E18.5)	n/a	↓↓ GlyT proportion of Jz	n/a	n/a	Reduced Lz size	(Kuhnel et al., 2017)
Peg3$^{+/-}$	Knockout*	↓ (E17.5) ↓ (P0)	↓↓ (E14.5, E17.5)	↓↓ glycogen (E14.5)	↓↓ (males more affected than females)	↓ *Tpbpa* (in males only) *Pcdh12* and *Gjb3* normal	n/a	n/a	(Lee et al., 2015, Thiaville et al., 2013, Kuroiwa et al., 1996, Relaix et al., 1996, He et al., 2016, Curley et al., 2004, Li et al., 1999, Tunster et al., 2018)
Phlda2$^{+/+BACx1}$ or *Phlda2*$^{+/+BACx3}$	2-4-fold over-expression of *Phlda2*	↓ (E18.5)	↓ (variable, from E12.5)	↓↓↓ glycogen ↓ PAS	n/a	↓↓↓ *Tpbpa* *Pcdh12* normal *Gjb3* normal	Impaired migration of PAS^{+} cells to decidua	n/a	(Tunster et al., 2010, Tunster et al., 2014)
Pik3ca$^{-/+}$ or *Pik3ca*$^{+/-}$	Knockout	↓ (E15.5)	↓ (E15.5, E18.5)	↓↓ glycogen (if dam is *Pik3ca*$^{+/-}$)	n/a	n/a	n/a	Reduction in Lz size, fetal capillary volume, exchange surface area and diffusion capacity dependent upon	(Foukas et al., 2006, Sferruzzi-Perri et al., 2016)

								maternal genotype	
Ptp4a2$^{-/-}$	Knockout	↓ (E16.5)	↓↓ (E16.5)	↓ PAS	↓ Non-migratory GlyT ↓↓↓↓ Migratory GlyT	↓ *Tpbpa*	Failure of *Tpbpa*$^{+}$; PAS^{+} cells to migrate to decidua	Reduced Lz size, Reduced transport capacity	(Dong et al., 2012)
Pthlh$^{-/-}$	Knockout	↓ (from E16.5)	Normal (E12.5, E14.5, E16.5, E18.5)	↓ glycogen (E12.5, E14.5, E18.5) normal glycogen (E16.5)	n/a	↓ *Tpbpa*	n/a	Reduced Lz size, Reduced transport capacity	(Duval et al., 2017)
Ldoc1$^{-/+}$ or *Ldoc1*$^{-/-}$	Knockout	↓ (E16.5) Normal (E18.5)	Normal (E12.5) ↑ (E16.5, E18.5)	n/a	Normal	*Pcdh12* normal	Mislocalisation of *Prl6a1*$^{+}$ cells in Lz	n/a	(Naruse et al., 2014)
Sp1$^{+/-}$;*Sp3*$^{+/-}$	Embryonic lethal between E16.5 and birth	↓↓ (E16.5)	↓ (E14.5)	n/a	n/a	↓ *Tpbpa* ↓ *Pcdh12*	n/a	Disorganised structure	(Kruger et al., 2007)
Tex19.1$^{-/-}$	Knockout	↓ (E18.5)	↓↓ (E18.5)	n/a	↓↓↓ (E18.5)	↓ *Tpbpa* ↓ *Pcdh12* ↓ *Gjb3*	n/a	Fewer S-TGCs	(Celebi et al., 2012, Reichmann et al., 2013)
Tfap2c$^{+/-}$	Knockout	↓ (E14.5) ↓ (E18.5)	↓ (E14.5, E18.5; trend only)	n/a	n/a	n/a	Mislocalisation of PAS^{+} cells within Lz	Disrupted and haemorrhagic Lz	(Kuckenberg et al., 2010, Kaiser et al., 2015)
Cre-Tpbpa:Tfap2c$^{-/-}$	Conditional knockout of *Tfap2c* in *Tpbpa*$^{+}$ cells	↓ (from E16.5)	n/a	↓↓ glycogen (E14.5)	↓↓↓↓ (E16.5)	↓ *Tpbpa* (E12.5) ↓↓↓↓ *Tpbpa* (E16.5) ↓ *Gjb3*	n/a	n/a	(Sharma et al., 2016)
Wdr13$^{-/+}$	Knockout*	↓ (from E17.5)	↓ (E17.5, E18.5)	↓ PAS staining	n/a	n/a	n/a	Reduced Lz size, Fewer S-TGCs, Increased MBS	(Singh et al., 2015)

↓ = <20% reduction; ↓↓ = 20-50% reduction; ↓↓↓ = >50% reduction; ↓↓↓↓ = undetected; ↑ = <20% increase; ↑↑ = 20-50% increase; ↑↑↑ = >50% increase. Developmental stage in brackets is the time assessed. Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. E, embryonic day; GlyT, glycogen trophoblast cells; Jz, junctional zone; Lz, labyrinth zone; MBS, maternal blood sinusoids; P, postnatal day; n/a, not available; SpT, spongiotrophoblast cells; S-TGCs, sinusoidal trophoblast giant cells; Tg, transgenic. * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.

Table 3: Genetic mouse models with fetal growth restriction and increased placental glycogen content and/or GlyT phenotypes

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localisation	Lz phenotype	References
Ascl2-Tg	*Ascl2* over-expression	↓ (E18.5)	↓ (E12.5, E14.5) Normal (E16.5, E18.5)	↑↑ glycogen (E16.5) ↑↑↑ glycogen (E18.5)	n/a	↓↓↓ *Tpbpa* ↑↑ *Prl7b1* Normal *Pcdh12* Normal *Gjb3*	Mislocalisation of *Tpbpa*$^{+}$; PAS^{+} cells within Lz	n/a	(Tunster et al., 2016)
Crim1$^{KST264/KST264}$	Hypomorphic generated by gene-trap insertion	↓ (E17.5)	↓ (from E13.5)	Normal PAS	n/a	↑ *Pcdh12*$^{+}$ cell area	n/a	Fewer S-TGCs	(Pennisi et al., 2007, Pennisi et al., 2012)
Csf2$^{-/-}$	C57BL/6 background	↓ (E14.5) Normal (E17.5)	↓ (E17.5)	n/a	↑↑↑	↑ *Tpbpa*	n/a	n/a	(Sferruzzi-Perri et al., 2009)
Esx1$^{-/+}$ or *Esx1*$^{-/-}$	Knockout*	↓↓ (P0)	↑↑↑ (E14.5) ↑↑ (E18.5)	n/a	↑	↑ *Tpbpa*	n/a	Vascularisation defect. Failure of SynT-II formation	(Li et al., 1997, Li and Behringer, 1998)
miR-126a$^{-/-}$	Knockout, Embryonic lethal (from E15.5)	↓ (E15.5)	Normal (E12.5, E15.5)	↑↑↑ glycogen (E15.5)	↑ (E13.5, E15.5)	↑ *Tpbpa* ↑ *Cdkn1c* (E15.5)	n/a	Increased Jz:Lz ratio (no effect on gross Lz size)	(Sharma et al., 2019)
Pcdh12$^{-/-}$	Knockout	↓ (E12.5, E17.5)	↓ (E17.5)	↑↑↑ glycogen (E12.5) ↑↑ glycogen (E17.5)	n/a	n/a	Mislocalisation of PAS^{+} cells within Lz	Reduced cell density	(Rampon et al., 2005, Rampon et al., 2008)
Plac1$^{-/+}$ or *Plac1*$^{-/-}$	Knockout*	↓ (E16.5)	↑↑↑ (E16.5)	↑ PAS	n/a	n/a	n/a	n/a	(Cocchia et al., 2000, Jackman et al., 2012)
Phlda2$^{-/+}$	Knockout*	Normal (vs. *Phlda2*$^{+/+}$ littermates) ↓ (vs. wild type litters)	↑↑ (from E12.5)	↑↑ glycogen	n/a	↑↑ *Tpbpa* Normal *Pcdh12* Normal *Gjb3*	n/a	n/a	(Frank et al., 1999, Frank et al., 2002, Tunster et al., 2015)
Prl7d1$^{-/-}$	Knockout	↓ (E12.5)	↓ (E12.5 in males)	↑↑↑ PAS (males only)	↑↑ (males	n/a	n/a	Increased Jz:Lz ratio. Impaired	(Zhang et al., 2019)

					only)			remodelling of maternal spiral arteries.	
Pthlh$^{-/-}$	Knockout	↓ (from E16.5)	Normal	↓ glycogen (E12.5, E14.5) Normal glycogen (E16.5) ↑ glycogen (E18.5)	n/a	↓ *Tpbpa*	n/a	Reduced Lz size, Reduced transport capacity	(Duval et al., 2017)

↓ = <20% reduction; ↓↓ = 20-50% reduction; ↓↓↓ = >50% reduction; ↑ = <20% increase; ↑↑ = 20-50% increase; ↑↑↑ = >50% increase. Developmental stage in brackets is the time assessed. ǂ Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; P, postnatal day; n/a, not available; S-TGCs, sinusoidal trophoblast giant cells; SynT-II, syncytiotrophoblast cell layer II; Tg, transgenic. * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.

Table 4: Genetic mouse models with normal or enhanced fetal growth and increased placental glycogen content and/or GlyT phenotypes

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localisation	Lz phenotype	References
Cdkn1c$^{-/+}$	Knockout*	↑ (E15.5, E18.5) = (P0)	↑↑↑ (from E13.5)	↓↓ glycogen (E15.5, E18.5)	Normal	↓ *Tpbpa* ↓ *Gjb3* *Pcdh12* normal	n/a	Increased Lz cell number, Fewer S-TGCs	(Georgiades et al., 2002, Coan et al., 2006, Tunster et al., 2011, Takahashi et al., 2000)
Cts7 ^{Tg}	*Cts7* over-expression	n/a	n/a	n/a	↓↓ (E12.5)	↓ *Tpbpa* (E10.5, E12.5)	n/a	Fewer S-TGCs	(Hemberger et al., 2000, Screen et al., 2008)
Egfr^{Dsk5}	*Egfr* hypomorph	Normal (E15.5)	↑ to ↑↑↑ (variable, E15.5) Genetic background dependent	↑ PAS	↑	↑ *Tpbpa* ↑ *Pcdh12*	n/a	↓ *Gcm1* ↓ *Dlx3*	(Dackor et al., 2007, Dackor et al., 2009b)
H19$^{-/-}$	Knockout*	↑ (from E15.5)	↑ (E15.5, E18.5)	↑ glycogen (E15.5)	↑↑ (E15.5)	n/a	n/a	n/a	(Leighton et al., 1995, Esquiliano et al., 2009)
Pgf$^{-/-}$	Knockout	Normal (P0)	↑ (P0)	↑↑ glycogen ↑ Best's Carmine staining	↑	n/a	n/a	Reduced Lz size	(Achen et al., 1997, Tayade et al., 2007, Parchem et al., 2018)

↓ = <20% reduction; ↓↓ = 20-50% reduction; ↓↓↓ = >50% reduction; ↑ = <20% increase; ↑↑ = 20-50% increase; ↑↑↑ = >50% increase. Developmental stage in brackets is the time assessed. ‡ Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; P, postnatal day; n/a, not available; S-TGCs, sinusoidal trophoblast giant cells; Tg, transgenic. * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.

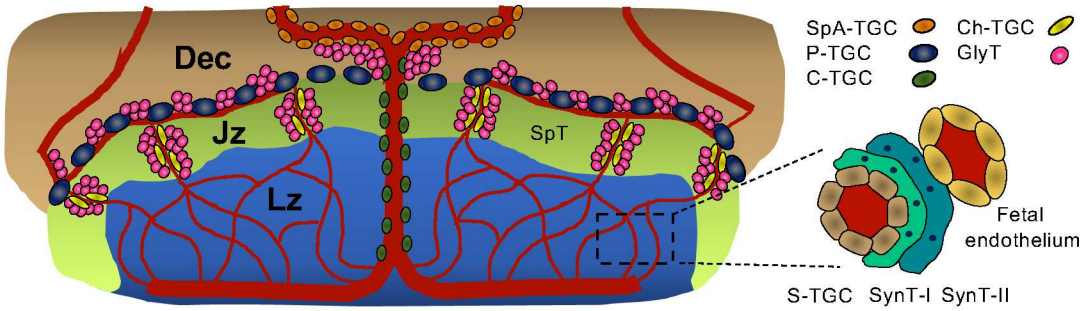
Table 5: Genetic mouse models with glycogen content or GlyT phenotypes associated with embryonic lethality at mid-gestation

Mouse model	Characteristics	Stage of embryonic lethality	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localisation	Lz phenotype	References
Arnt$^{-/-}$	Knockout	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E9.5)	↓↓↓↓ *Tpbpa* (E9.5)	n/a	Reduced Lz size, Labyrinth trophoblast defect, decreased VEGF expression	(Adelman et al., 2000, Kozak et al., 1997, Maltepe et al., 1997, Watson and Cross, 2005)
Ascl2$^{-/-}$ or *Ascl2*$^{-/+}$	Knockout*	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E8.5)	↓↓↓↓ *Tpbpa* (E8.5)	n/a	Vascularisation defect	(Guillemot et al., 1995, Guillemot et al., 1994, Rossant et al., 1998)
Cited2$^{-/-}$	Knockout; associated with congenital malformations (e.g., NTD)	By E13.5	↓↓ (E12.5)	n/a	↓ Migratory GlyT	↓ *Tpbpa*	Impaired migration to decidua	Vascularisation defect	(Dunwoodie et al., 1998, Withington et al., 2006, Yin et al., 2002, Barbera et al., 2002)
Hectd1$^{-/-}$	Knockout; associated with congenital malformations (e.g., heart defects)	By E12.5	↓ (incomplete penetrance)	↓↓ glycogen	↓	↓ *Tpbpa* ↓ PAS	n/a	Disrupted structure, Vascularisation defect	(Sarkar et al., 2014, Sarkar et al., 2016)
Hif1a$^{-/-}$	Knockout; associated with congenital malformations (e.g., NTD, heart defects)	By E10.5	n/a	n/a	↓↓↓ progenitors	↓ *Tpbpa*	n/a	Impaired vascularisation of chorion	(Cowden Dahl et al., 2005, Kozak et al., 1997, Compernolle et al., 2003)
Pdcd5$^{-/-}$	Knockout; associated with congenital malformations (e.g., heart defects)	By E13.5	n/a	↓↓↓↓ PAS (E13.5)	↓↓↓↓ (E13.5)	n/a	n/a	Disorganised structure, Necrotic and haemorrhagic	(Li et al., 2017)
Peg10$^{+/-}$	Knockout*	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E9.5)	↓↓↓↓ *Tpbpa* (E9.5)	n/a	Impaired development	(Ono et al., 2006)

Prdm1$^{-/-}$	Knockout; associated with congenital malformations (e.g., heart defects)	By E10.5	n/a	n/a	n/a	Normal *Tpbpa*	Failure to migrate to decidua	Disrupted structure	(Mould et al., 2012, Vincent et al., 2005)

↓ = <20% reduction; ↓↓ = 20-50% reduction; ↓↓↓ = >50% reduction; ↓↓↓↓ = undetected; Developmental stage in brackets is the time assessed.

ǂ Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; n/a, not available; * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.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GlyT
Fetal
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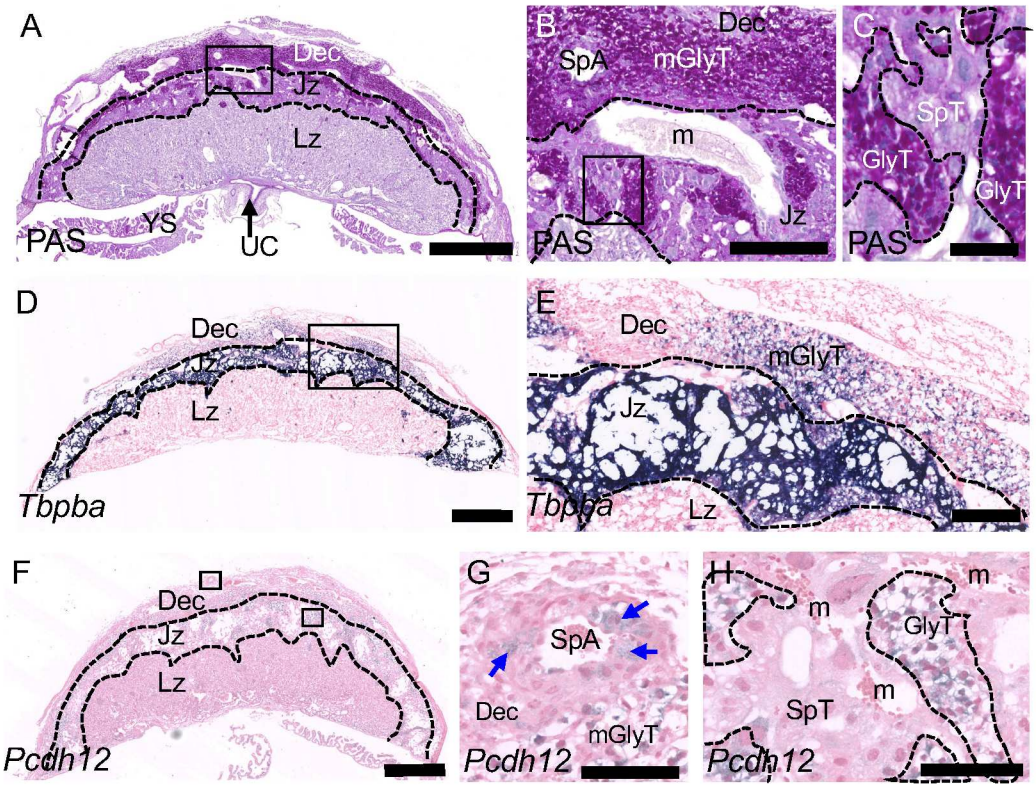
S-TGC
SynT-I
SynT-II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